Chromatin Insulator Elements Block the Silencing of a Target Gene by the Drosophila Polycomb Response Element (PRE) but Allow *trans* Interactions Between PREs on Different Chromosomes

Christian J. A. Sigrist and Vincenzo Pirrotta

Department of Zoology, University of Geneva, CH1211 Geneva, Switzerland

Manuscript received March 14, 1997
Accepted for publication June 2, 1997

ABSTRACT

Polycomb response elements (PREs) can establish a silenced state that affects the expression of genes over considerable distances. We have tested the ability of insulator or boundary elements to block the repression of the *miniwhite* gene by the *Ubx* PRE. The gypsy element and the scs element interposed between PRE and *miniwhite* gene protect it against silencing but the scs′ is only weakly effective. When the PRE-*miniwhite* gene construct is insulated from flanking chromosomal sequences by gypsy elements at both ends, it can still establish efficient silencing in some lines but not others. We show that this can be caused by interactions in *trans* with PREs at other sites. PRE-containing transposons inserted at different sites or even on different chromosomes can interact, resulting in enhanced silencing. These *trans* interactions are not blocked by the gypsy insulator and reveal the importance of nonhomologous associations between different regions of the genome for both silencing and activation of genes. The similarity between the behavior of PREs and enhancers suggests a model for their long-distance action.

THE transcriptional potential of many Drosophila genes is governed by the assembly of chromatin complexes that affect the ability of neighboring genes to respond to activators. The best known case is that of the homeotic genes whose expression in inappropriate segmental domains is prevented by the formation of complexes involving the products of the Polycomb group of genes (PcG). These proteins assemble cooperatively beginning at certain regulatory regions, the Polycomb response elements (PREs), and repress enhancers or promoters over an extended region, which, in the *Ubx* gene, can involve many tens of kilobases (PIRROTTA 1995). A simple way to monitor the formation of such complexes is to test the effects of a PRE on the eye pigmentation dependent on the expression of a *miniwhite* gene contained in the same transposon. This has been shown to reflect the repression of other reporter genes present in the transposon (FAUVARQUE and DURA 1993; CHAN *et al.* 1994; ZINK and PARO 1995). In general PcG activity results in the partial repression of the *miniwhite* gene and a variegated eye pigmentation, frequently in clonal patches, highly reminiscent of the position effect variegation obtained when a *white* gene is placed in the vicinity of heterochromatin (FAUVARQUE and DURA 1993; CHAN *et al.* 1994; KASSIS 1994; GINDHART and KAUFMAN 1995). These effects, the genetic and the structural similarities found between PcG proteins and heterochromatin proteins (the products of the *Suvar* genes), have suggested that both PcG and heterochromatic silencing have a similar molecular basis and that they are caused by chromatin complexes that assemble cooperatively and spread to involve extended chromosome regions. A variety of experiments suggest that, although such complexes initiate at PREs, they involve sequences in the surrounding regions. Chromatin cross-linking experiments indicate that, in a repressed or silenced *Ubx* gene, Polycomb protein spreads beyond the restriction fragments containing known PREs (ORLANDO and PARO 1993). Sequences surrounding the insertion site of a transposon containing a PRE have a strong influence on the degree of repression that can be established since the same transposon can show a strong degree of silencing or variegation at one chromosomal site but not at another. Pairing of complexes formed at PREs on two homologous chromosomes greatly strengthens the degree of silencing (FAUVARQUE and DURA 1993; KASSIS 1994; KAPOUN and KAUFMAN 1995). Evidence of this type led to the formulation of a model for PcG complex formation in which an initial core, assembled at the PRE, extends and stabilizes itself by recruiting frequently occurring but isolated and weaker target sites for PcG proteins in the surrounding regions (PIRROTTA and RASTELLI 1994). According to this model then an effective silencing complex requires the participation of regions surrounding the PRE, which acts as an initiator of the silencing complex.

The effect of PRE-initiated silencing on adjacent genes is potentially dangerous if it cannot be prevented from spreading to genes that should not be silenced. We might expect therefore that some mechanism exists

Corresponding author: Vincenzo Pirrotta, Department of Zoology, University of Geneva, 30 quai Ernest Ansermet, CH1211 Geneva, Switzerland. E-mail: pirrotta@sc2a.unige.ch

to contain such spreading. In the past few years, three types of sequence elements have been identified that act as barriers or insulators with respect to enhancer-promoter interactions. These are the scs and scs′ elements, originally found flanking a region containing two *hsp-70* genes (KELLUM and SCHEDL 1991), and the gypsy element, a fragment from the *gypsy* transposon containing a set of binding sites for the su(Hw) protein (GEYER and CORCES 1992). These three elements, although they bind different proteins, have very similar properties: when placed between enhancer and promoter they prevent the activation of the promoter by the enhancer but have no effect when placed just on the outside of the enhancer-promoter interval (HOLDRIDGE and DORSETT 1991; KELLUM and SCHEDL 1992; ROSEMAN *et al.* 1993; CAI and LEVINE 1995; SCOTT and GEYER 1995). This directional effect, whose molecular basis is not understood, gives them insulator-like properties. The scs′ element contains binding sites for the BEAF-32 protein, which have been shown to be necessary for its insulator activity (ZHAO *et al.* 1995). The best known of the three elements is however the gypsy su(Hw) target region. The su(Hw) protein contains multiple zinc finger motifs and binds to 12 sites in a 400 base pair (bp) interval within gypsy, which is sufficient to give insulator activity (MAZO *et al.* 1989; SPANA and CORCES 1990; GEYER and CORCES 1992). In this paper we will refer to this fragment as the gypsy element. Mutations in the *su(Hw)* gene cause loss of the insulator activity of the gypsy element, making it therefore a particularly useful tool since the behavior of a particular construct can be tested in permissive and nonpermissive conditions.

Some evidence suggests that insulator elements could block repressive chromatin effects as well as prevent the interaction of enhancers with promoters. A transposon containing a *white* gene insulated on both sides by the gypsy element is partially shielded against variegation effects when inserted in heterochromatin (ROSEMAN *et al.* 1993). This suggested that insulator elements might also prevent the silencing effects of a PRE. In this article we show in fact that insulator elements inserted between PRE and target gene prevent the silencing of that gene. We have then used such elements to insulate a construct containing a PRE plus *miniwhite* gene from surrounding sequences to determine the importance of the participation of flanking sequences in the establishment of the repressed state by the PRE. The results show that the gypsy and scs insulators block completely the silencing effect of the PRE. Although the flanking sequences are important, the PRE is still able to silence even when insulated from their direct contribution by gypsy elements on both sides. Surprisingly, however, it continues to behave differently at different chromosomal sites. We show that this is most likely due to pairing interactions with other chromosomal sites on the same or different chromosomes.

MATERIALS AND METHODS

Transposon construction: The PGM, PSM and PS′M transposon constructs were assembled in the CaSpeR1 vector (PIRROTTA 1988) while CaSpew 15, a precursor of the CaSpeR vector containing *Eco*RI sites on both sides of the *miniwhite* gene, was used for the GPM and GPMG constructs. The PRE used was a 661-bp *Nde*I-*Pst*I fragment containing the core activity of the major *Ubx* PRE, located ~24 kilobases upstream of the *Ubx* promoter (CHAN *et al.* 1994). The gypsy element was a 436-bp *Ball*-*Bst*XI fragment from the gypsy transposon, obtained from P. GEYER, containing 12 binding sites for the su(Hw) protein (GEYER and CORCES 1992). The scs element was a 1.8-kb *Bam*HI-*Bgl*II fragment (KELLUM and SCHEDL 1991) obtained from F. KARCH. The scs′ element was a 454-bp *Eco*RI-*Bam*HI fragment containing the core scs′ element (FARKAS and UDVARDY 1992; ZHAO *et al.* 1995), obtained from U. LAEMMLI. The fragments, cloned in pUC18 or Bluescript vectors, were excised with appropriate restriction enzymes and inserted in the CaSpeR1 polylinker, immediately upstream of the *miniwhite* gene. The GPM and GPMG constructs were made by first eliminating the *Eco*RI site upstream of the *miniwhite* gene in the CaSpew 15 vector by partial *Eco*RI digestion and filling the ends with Klenow DNA polymerase. The upstream fragments were then inserted in appropriate sites in the polylinker and the downstream gypsy element in GPMG was inserted in the remaining *Eco*RI site at the 3′ end of the *miniwhite* gene. To make the YGPMG construct, the gypsy-PRE-*miniwhite*-gypsy fragment excised from GPMG was inserted in C4-yellow, a Carnegie 4 transposon vector (RUBIN and SPRADLING 1983) containing the 5.2-kb intronless *yellow* gene (GEYER and CORCES 1992) inserted in the *Sal*I site.

Fly strains: All constructs were injected into the host strain $Df(1)w^{67c23}$. The response to PcG mutations was tested using the dominant effect mutation Psc^{1}, which acts as a gain of function mutation that increases repression, and the $Su(z)2^{1}$ mutation, a dominant suppressor of PcG repression (WU and HOWE 1995; L. RASTELLI, personal communication). To test for heterochromatic position effects we used the $Suvar(2)5^{1}$ mutation in the gene encoding the HP1 heterochromatin protein. The effect of temperature on the variegation was also used since in our experiments PcG repression always increases at higher temperature while heterochromatic PEV decreases. The lines were tested in a $su(Hw)^{-}$ background by crossing males to female $y^{2}\ sc\ ct^{6}\ f^{1}\ w^{67}$; $bx^{34e}\ su(Hw)^{v}/TM6\ su(Hw)^{f}\ Ubx$, selecting male progeny and backcrossing to the same strain to obtain $su(Hw)^{-}$ progeny, identified by the suppression of the gypsy-induced cut mutation. For lines with insertions on the *X*, the *TM3* balancer third chromosome was first introduced in the line, and then females were crossed with the *su(Hw)* strain, progeny carrying the *TM3* balancer were selected and backcrossed to the *su(Hw)* strain to obtain the $su(Hw)^{-}$ flies. The $mod(mdg4)^{u1}$ mutant was obtained from T. GERASIMOVA and crossed into a w^{-} background.

Transformed lines: The transformed lines were examined by Southern blot hybridization to check for the integrity of the transposon and the number of copies present. In a number of lines the insertion site was determined by *in situ* hybridization to salivary gland chromosomes. The presence of eye variegation was determined visually and the eyes were photographed with a camera mounted on a Wild MP9 microscope using flies raised at 25° and aged 2 days.

RESULTS

The gypsy element blocks silencing by the PRE: A 661-bp *Pst*I-*Nde*I PRE core fragment, inserted in a CaSpeR vector, generally causes variegated *miniwhite*

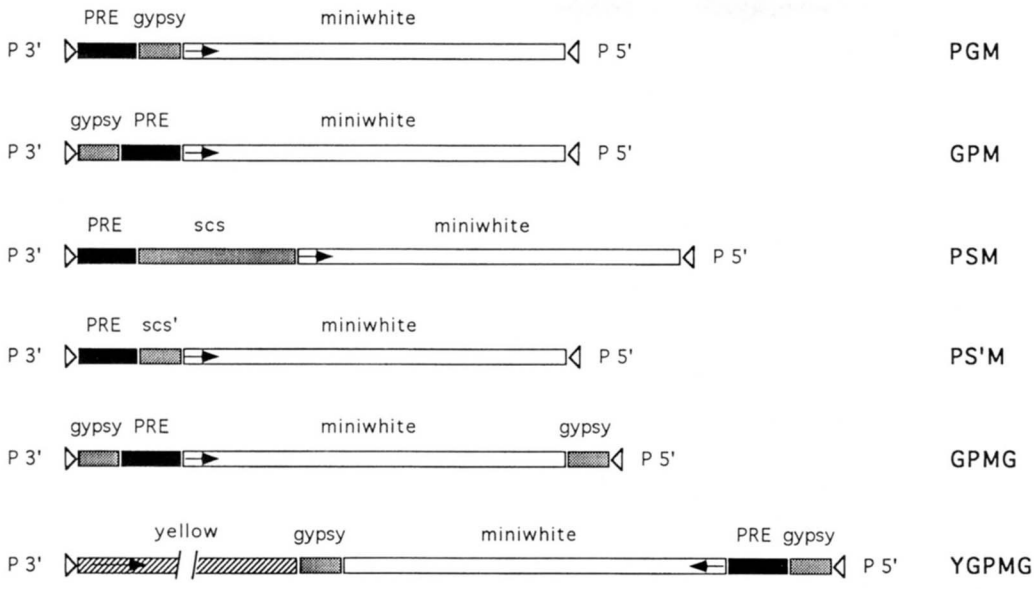

FIGURE 1.—Transposon constructs. The constructs are shown schematically with the PRE, gypsy, scs, scs′, *miniwhite* and *yellow* fragments indicated. The arrow shows the direction of transcription of the *miniwhite* and *yellow* genes. The *yellow* gene is not shown in scale.

gene expression in 50–60% of the lines and this variegation is dependent on PcG genes (CHAN *et al.* 1994 and unpublished observations). When we inserted the gypsy su(Hw) binding region between the PRE and the *miniwhite* gene (PGM construct, Figure 1), only one of the 31 PGM lines obtained showed detectable variegation. Such a low frequency is obtained with transposons that do not contain a PRE and, since the single variegating line obtained is suppressed by *Suvar(2)5*^{1}, a typical suppressor of heterochromatic position-effect variegation (PEV), it is probably due to a heterochromatic insertion (not shown). To test whether the mere presence of the gypsy element interferes with the establishment of a repressive complex at the PRE, we constructed a transposon with the configuration gypsy-PRE-*miniwhite* (GPM construct, Figure 1). Of the 19 single insert lines obtained with this construct, 14 showed eye variegation in the heterozygous or homozygous state, showing that the vicinity of the gypsy element does not by itself interfere with the function of the PRE. We conclude that silencing is blocked only when the gypsy element is interposed between the PRE and the reporter gene and, just as in the enhancer-blocking action, its effect is that of an insulator (GEYER *et al.* 1986; GEYER and CORCES 1992).

The GPM lines also illustrate the previously observed phenomenon of pairing dependence: the repressive effect of the PRE is often dramatically enhanced in flies homozygous for a transposon insertion (KASSIS *et al.* 1991; FAUVARQUE and DURA 1993). In seven of the 14 variegating lines, the homologous pairing of the two PRE-containing transposons causes a complete repression of the *miniwhite* gene and a completely white eye. In some cases, this is observed even when no variegation was detected in the heterozygous flies.

We also tested the scs and scs′ insulators for ability to block the silencing effect of the PRE. These elements were inserted between the PRE and the *miniwhite* gene to generate the PSM and PS′M constructs (Figure 1). Of the 38 transgenic lines obtained with the PSM construct, none showed eye variegation, indicating that the scs element also acts as a strong insulator against PRE repression. For the PS′M construct, we obtained 28 lines, nine of which show eye variegation. One of these variegated in the heterozygous state while the others variegated only when homozygous. Both the overall frequency of variegation and the proportion of lines that variegate in the heterozygous state are distinctly lower than the ~50% and 25% expected in the absence of an insulating activity. In addition, the degree of repression is generally lower than that obtained in the absence of insulator and in no case results in completely white eyes in flies homozygous for the transposon. The results suggest that the scs′ element behaves differently from gypsy and scs, either because it is intrinsically a weaker insulator or because it acts by a different mechanism that interferes less with the PRE silencing activity.

Blocking action is dependent on *su(Hw)*: To prove that the lack of eye color variegation in the PGM lines is due to the blocking effect of the interposed gypsy element, 12 of the lines carrying this construct were crossed into a *su(Hw)*$^{-}$ background. The choice of the lines to be so tested, in this and other cases below, was not arbitrary but dictated by the fact that the *su(Hw)* gene is on the third chromosome and the crosses can be conveniently done only with lines carrying insertions on the *X* or second chromosome. The results, summarized in Table 1, reveal that in the absence of *su(Hw)* function eight of the 12 lines variegate, approximately the frequency expected in the absence of an insulator. In contrast, the majority of the 12 GPM lines tested are not visibly affected by the *su(Hw)* mutation. The variegating lines continue to variegate in a *su(Hw)*$^{-}$ background and the lines that did not variegate remain

TABLE 1

PGM lines on chromosomes *X* and *2*

Line	Chromosome	wt background		*su(Hw)*$^-$ background		*mod(mdg4)*$^-$ background	
		T/+	T/T	T/+	T/T	T/+	T/T
PGM-15	*2*	–	–	–	–	–	–
PGM-19	*2*	–	–	–	White	–	+
PGM-21	*2*	–	Lethal	White	ND	+	Lethal
PGM-273	*2*	–	–	–	+	–	+
PGM-43	*2*	–	–	+	+	+	+
PGM-45	*2*	–	Lethal	–	Lethal	ND	ND
PGM-51	*2*	–	–	–	–	–	–
PGM-62	*2*	–	–	White	ND	+	ND
PGM-67	*2*	–	–	–	–	–	–
PGM-56	*X*	–	–	–	White	–	+
PGM-16	*X*	–	–	–	+	–	+
PGM-272	*X*	–	–	Males +	White	Males +	+

The columns show the line name, the chromosome in which the transposon is inserted and the behavior of the transposon in the heterozygous or homozygous state in a wild-type, in a *su(Hw)*$^-$ or in a *mod(mdg4)*$^-$ background. Lines on chromosome *3* were not tested in the mutant backgrounds. The presence or absence of variegation are indicated by a + or −. Strong silencing causing complete repression is indicated by "white" and homozygous lethality by "lethal." *ND,* not done.

nonvariegating, indicating that the mere proximity of the gypsy element does not in itself interfere with PRE-dependent repression. In two lines, the loss of *su(Hw)* function causes a decrease in variegation and, in another line, variegation appears where none was detectable before. We interpret these as due to contributions of flanking sequences revealed by the lifting of the insulation.

In several PGM lines, the lifting of the insulation reveals complete silencing of the *miniwhite* gene, resulting in a total lack of eye pigmentation (Figure 2A and C). Two of the 12 lines tested reveal complete repression already when heterozygous for the transposon. Since eye pigmentation is used to identify transformed flies, such lines would not be recoverable were it not for the conditional insulator and their existence indicates that the frequency of variegation is normally underestimated. Though the numbers are too low for good statistics, they suggest that some 20% of the insertions containing this particular PRE would go undetected because the *white* gene is completely repressed.

Mutations in another gene called *mod(mdg4)* also affect the insulating activity of the gypsy element. *mod(mdg4)* mutations relieve the block caused by gypsy insertions in some genes but in other cases, in particular, in the *yellow* gene, they silence enhancers on both sides of the gypsy insertion (GERASIMOVA *et al.* 1995; GEORGIEV and KOZYCINA 1996). When we tested the PGM lines in a *mod(mdg4)*$^-$ background, we found that the effects paralleled those of *su(Hw)*$^-$ mutations (Table 1). In all cases the result was a release of the gypsy barrier and partial silencing of the *miniwhite* gene, though always to a lesser extent than in the *su(Hw)*$^-$ background. *mod(mdg4)* mutations are also said to have a *trithorax*-like effect reducing the expression of homeotic genes as well as enhancing heterochromatic position effect variegation. To determine whether the *mod(mdg4)* mutation acts on the gypsy insulator or simply affects the expression of the *miniwhite* gene, we tested also lines carrying the GPM transposon or a *miniwhite* gene with neither gypsy element nor PRE. In the GPM case, only three of the 12 lines tested showed any effect of *mod(mdg4)* and then always in the same sense but weaker than the effect of *su(Hw).* No effect was seen in the absence of gypsy or PRE, indicating that *mod(mdg4)* does not directly affect *miniwhite* expression or PRE silencing as such but most likely is required for effective insulation by the gypsy element.

Insulation from the chromosomal context: A prominent feature of PRE silencing is its variability and strong dependence on the chromosomal context. As previously observed and as illustrated by the foregoing experiments, the occurrence and degree of silencing of the same PRE-containing construct depend on the site of insertion. This variability implies that the sequences flanking the transposon insertion or the chromosomal site make an important contribution to silencing that might be either positive or negative. The different degrees of repression are reflected by the ability of PcG proteins to bind to a given PRE-containing transposon at different insertion sites (ZINK *et al.* 1991). A transposon insertion that results in strong silencing is accompanied by the creation of a new chromosomal binding site for PcG genes detectable in polytene chromosomes. Insertions that do not result in silencing do not create new PcG binding sites. Since the gypsy element acts as an effective block for PRE action, we constructed a transposon in which the PRE-*miniwhite* gene is insulated

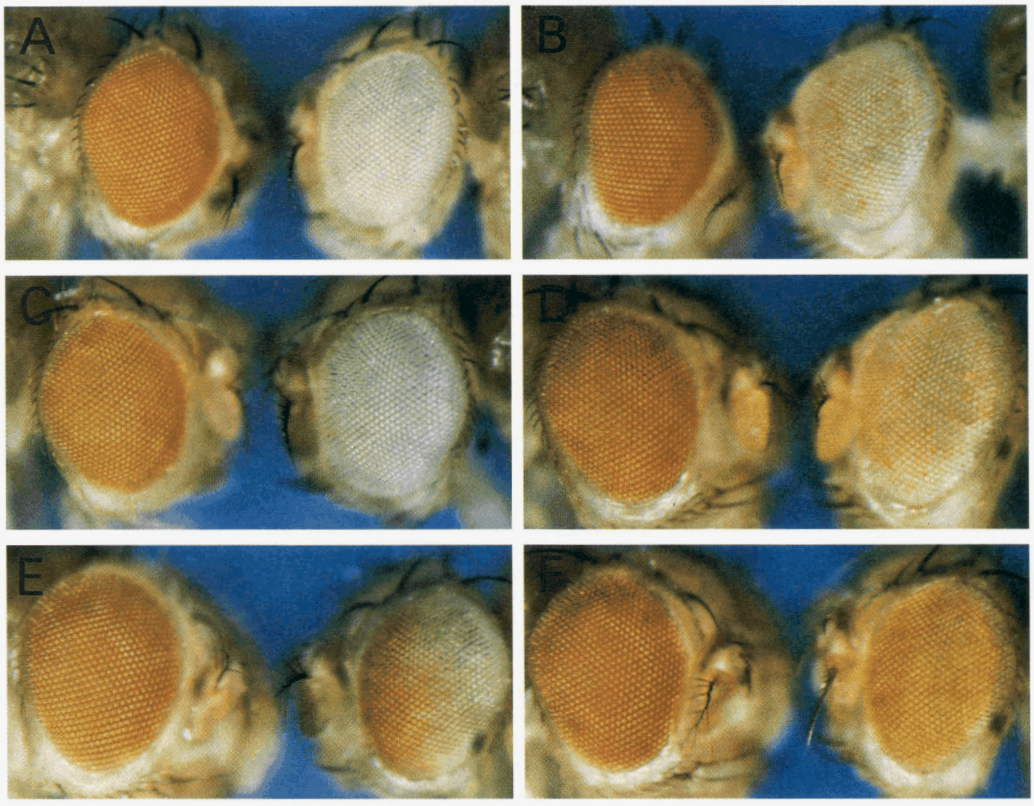

FIGURE 2.—Variegation of PGM lines. No variegation was found in 30 of 31 PGM lines but many lines become variegated when placed in a *su(Hw)*$^-$ background. (A) PGM-21 males with one copy of the transposon in a wild-type (left) or *su(Hw)*$^-$ background (right). (B) PGM-21 males with one copy of the transposon in a wild-type (left) or *mod(mdg4)*$^-$ background (right). (C) PGM-272 females with two copies of the transposon in a wild-type (left) or a *su(Hw)*$^-$ (right) background. (D) PGM-272 females with two copies of the transposon in a wild-type (left) or *mod(mdg4)*$^-$ background (right). (E) PGM-273 females homozygous for the transposon in a wild-type (left) or *su(Hw)*$^-$ background (right). (F) PGM-273 females homozygous for the transposon in a wild-type (left) or *mod(mdg4)*$^-$ background (right).

on both sides by gypsy elements, to test the intrinsic effects of the PRE on *miniwhite* gene expression.

We obtained 17 lines carrying a single copy of such a doubly blocked transposon, GPMG (Figure 1). We note first that these lines, though less pigmented on average than a nonblocked CaSpeR transposon, range in eye color from pale yellow to red-orange. This was unexpected because the insulation of a *miniwhite* gene from the chromosomal context has previously been shown to render it context independent and to decrease its expression, which normally relies on inductive effects from distant enhancers for high levels of *miniwhite* activity (KELLUM and SCHEDL 1991; ROSEMAN *et al.* 1993). Since genomic Southern blots verified that these lines contained single insertions, this suggests that the *miniwhite* gene is not fully insulated by the flanking gypsy elements. Table 2 shows that none of the 17 GPMG lines obtained variegates in the heterozygous state except one in which very faint variegation develops with age. However, when the flies are homozygous for the transposon, 12 lines show variegation or complete repression, one does not and four could not be tested because they are homozygous lethal. When nine of the lines (all those with insertions on the *X* or second chromosome) are placed in a *su(Hw)* mutant background, one now variegates as heterozygote. The strong repression observed in the homozygous state persists in the *su(Hw)* background except for one line, which reveals variegated pigmentation instead of being completely white.

The very low frequency of variegation in the heterozygous state raised the possibility that many lines were lost because the doubly insulated construct was often completely repressed. To determine this we generated transgenic lines containing the same GPMG construct in a transposon that used the *yellow* gene as a transformation marker. In this transposon, YGPMG (Figure 1), the *yellow* gene is outside the gypsy insulators and should not be affected by the PRE, allowing us to detect lines in which the *miniwhite* gene is completely repressed even in heterozygous state. Of 22 lines obtained with this construct, six are completely repressed as heterozygotes and would not have been detected in the absence of the *yellow* marker. When homozygous, the YGPMG lines confirm the GPMG results: six additional lines become completely white and three become variegated.

These results show that the PRE functions effectively

TABLE 2

GPMG lines

Line	Chromosome	wt background			su(Hw) background	
		T/+		T/T	T/+	T/T
GPMG-4	*2*	Orange	−	White	−	White
GPMG-6	*2*	Orange	−	Lethal	−	Lethal
GPMG-12	*X*	Red orange	−	White	−	+
GPMG-14	*2*	Dark orange	−	Lethal	−	Lethal
GPMG-16	*3*	Yell. orange	−	White	ND	ND
GPMG-18	*2*	Yell. orange	±	Lethal	White	Lethal
GPMG-182	*3*	Orange	−	White	ND	ND
GPMG-19	*3*	Orange	−	White	ND	ND
GPMG-21	*3*	Yell. orange	−	White	ND	ND
GPMG-27	*3*	Dark orange	−	White	ND	ND
GPMG-28	*2*	Orange	−	White	−	White
GPMG-30	*3*	Yell. orange	−	Lethal	ND	ND
GPMG-423	*2*	Orange	−	+	−	−
GPMG-43	*2*	Red	−	−	−	−
GPMG-50	*2*	Yell. orange	−	White	−	White
GPMG-54	*3*	Orange	−	White	ND	ND
GPMG-57	*3*	Dark orange	−	White	ND	ND

The columns show the line designation, the chromosome in which the transposon is inserted and the behavior of the transposon in the heterozygous or homozygous state either in a wild-type or in a *su(Hw)*$^-$ background. The eye color is indicated for the heterozygous state and + or − symbolize the presence or absence of variegation. Strong silencing causing complete repression is indicated by "white". Homozygous lethality is indicated by "lethal." ND, not done (when the transposon was on the third chromosome).

to repress the *miniwhite* gene even when prevented from interacting with flanking sequences. However, the very different levels of expression of the *miniwhite* and/or repression by the PRE observed in the different lines argue for the persistence of strong position effects despite the flanking gypsy insulators. Either the insulation by the gypsy element is incomplete to a degree not revealed by the PGM construct and previous reports or there exist other types of interaction that bypass the double gypsy block. One such type of interaction is that occurring in *trans* between two paired copies of GPMG. The pairing effect suggests the possibility that *trans* interactions might occur between nonhomologous genomic sites containing PREs.

Nonhomologous *trans* interactions: The pairing effect of GPMG transposons indicates that the gypsy insulator blocks only the propagation of effects along a chromatin strand but does not interfere with lateral interactions between two different chromatin strands. If the folding of chromosomes within the nucleus allowed two different PRE-containing sites to come in contact, they might also interact in *trans*, causing stronger silencing. To test whether such *trans* interactions could operate between two PREs at heterologous sites, we crossed the different GPMG lines in pairwise combinations and determined the degree of silencing of the *trans*-heterozygotes (Table 3). Interactions were found in a number of combinations, generally between lines that show strong silencing as homozygotes. However, strong homozygous silencing was no guarantee of interaction with another homozygous silencer. Frequently the *trans* interacting transposons are inserted relatively close to one another: GPMG-27 is at 62A, GPMG-30 is at 62A and GPMG-54 is at 67A-B. However, we found interactions between insertion sites quite distant from one another on the same chromosome: GPMG-4 is at 55B while GPMG-18 is at 46D and GPMG-28 is at 60D. In a few cases, interactions were observed between transposons on different chromosomes, for example between GPMG-27 on chromosome *3* and GPMG-28 on chromosome *2* (Table 3 and Figure 3, A and B). Interestingly, the lines that show *trans* interactions often form a commuting group: if A interacts with B and B interacts with C, A is likely also to interact with C, suggesting that the site of insertion contains sequences that favor pairing with PRE-containing sites. *Trans* interactions between nonhomologous sites are not fully competed by the homologous pairing of the chromosomes. Flies homozygous for GPMG-28 and heterozygous for GPMG-27 are less pigmented than flies heterozygous for GPMG-27 but slightly more than flies with one copy of each transposon (Figure 3, C and D). Flies doubly homozygous are entirely white-eyed (Figure 3E). This implies that the interactions are not entirely exclusive since pairing with the homologous insertion does not prevent the lightening effect on the nonhomologous insertion. As previously observed with other PRE-containing transposons (L. RASTELLI and V. PIRROTTA, unpublished observations), even the pairing with the homologous chromosome carrying no transpo-

TABLE 3

Interactions between GPMG lines

	Chromosome *3* lines									
GPMG	16	182	19	21	27	30	54	57	Y-60	Y-100
16	White									
182	−	White								
19	−	−	White							
21	−	+	−	White						
27	−	−	−	−	White					
30	−	+/−	−	−	White	Lethal				
54	−	−	−	−	+	+	White			
57	+/−	+/−	−	+/−	−	−	−	White		
Y-60	+/−	+	−	+	+	+	+	+	White	
Y-100	+/−	+	−	+	White	White	+	+	ND	White

	Chromosome 2 lines							
GPMG	4	6	14	18	28	423	43	50
4	White							
6	−	Lethal						
14	−	−	Lethal					
18	White	−	−	Lethal				
28	+	−	−	+	White			
423	−	−	−	−	−	+		
43	ND	−	−	−	−	−	−	
50	−	−	−	−	−	−	−	White

	Interchromosome crosses		
GPMG	4	18	28
27	T/T = T	T/T > T	T/T < T
30	T/T = T	T/T = T	T/T = T
54	T/T > T	T/T > T	T/T = T

GPMG lines containing insertions at different sites on the same chromosome were tested for interactions between *trans* heterozygotes. A combination was said to interact (+) when the *trans* heterozygotes were less pigmented than the stronger of the two heterozygotes separately. The chromosome *3* lines were also tested in *trans*-heterozygous crosses with two lines of YGPMG that are completely repressed as heterozygotes (Y-60 and Y-100). Complete repression is indicated by "white." ND, not done. In the last panel, the strongly interacting chromosome *2* lines are crossed with strongly interacting chromosome *3* lines. The *trans* heterozygous combination (T/T) is compared with the stronger of the two transposons separately (T).

son increases the degree of silencing, suggesting that the association with chromosomal sequences on the homologous chromosome contributes to silencing. Flies doubly heterozygous for GPMG-27 and GPMG-28 are less repressed when the pairing with the homologous chromosome is reduced by balancer chromosomes (Figure 3F). In addition, we tested combinations of GPMG insertions on chromosome *3* with two third-chromosome YGPMG insertions that are completely silenced even as heterozygotes (Table 3). Both YGPMG lines induce strong silencing of all the GPMG insertions tested but one, in two cases producing totally white eyes, suggesting that the YGPMG transposon is so strongly repressed in these two lines just because it has inserted in a site that interacts strongly with other PRE-containing sites in the genome.

Nonhomologous *trans* interactions are not limited to doubly blocked PRE constructs. We found frequent *trans* effects between GPM constructs (Table 4), arguing against a model in which the inability of a PRE to interact with flanking sequences enhances its tendency to interact with heterologous sites. However, we have not yet detected strong nonhomologous *trans* interactions between transposons containing the PRE but no gypsy. Does the gypsy insulator contribute to the *trans* interactions? Homologous pairing interactions between PREs are independent of gypsy and have been observed with many PRE-containing constructs (FAUVARQUE and DURA 1993; CHAN *et al.* 1994; KASSIS 1994; GINDHART and KAUFMAN 1995; KAPOUN and KAUFMAN 1995). The homologous pairing effect of GPMG transposons is not affected by mutation of *su(Hw)* (Table 2 and Figure 4, A and B). However, the *trans* interaction between the two pairs of GPMG lines tested, GPMG-4/GPMG-28 and GPMG-4/GPMG-18, is lost or greatly weakened in a *su(Hw)* background (Figure 4, C and D). This suggests that the su(Hw) complex formed at the gypsy insulator contributes to the *trans* interactions, although we can-

TABLE 4

Interactions between GPM lines

	Chromosome *3* lines							
GPM	12	331	49	69	85			
12	White							
331	+	White						
49	−	−	White					
69	++	+	+	++				
85	−	−	−	+	+/−			
	Chromosome *2* lines							
GPM	90	15	20	40	54	760	93	123
90	White							
15	+	+						
20	−	−	Lethal					
40	−	−	−	+				
54	−	−	−	++	Lethal			
760	−	−	−	−	−	−		
93	=	−	−	−	−	−	White	
123	++	+	−	+	+	−	−	White
	X chromosome lines							
GPM	91	330	39	71	761	105		
91	White							
330	−	White						
39	−	−	+					
71	−	−	−	−				
761	−	−	−	−	−			
105	−	+	−	−	−	+		

GPM lines containing insertions at different sites on the same chromosome were crossed. A combination was said to interact (+) when the *trans* heterozygotes were less pigmented than the darker of the two heterozygotes separately. Strong *trans* interactions (++) were scored when the *trans* heterozygote was less pigmented than either of the two parents. When the *trans* heterozygote had the same color as the two parents it was scored as =. Complete repression is indicated by "white."

not exclude that the loss of the insulation allows flanking sequences to interfere with PRE activity or alternatively that the removal of the gypsy block allows the PRE to interact with flanking sequences weakening the *trans* interactions with other sites.

DISCUSSION

Blocking the PRE: These experiments demonstrate that at least two of the three major insulating elements known, the gypsy and scs elements, can act as barriers not only to the interaction of enhancers and promoters but also to the interaction of silencing elements and their targets. The scs′ element probably has some insulating effect on the PRE but this activity is much weaker than that of the other two. VAZQUEZ and SCHEDL (1994), who used the same scs′ fragment, also found that it had only a weak effect as a blocker of activation of the *white* gene by the eye enhancer. The difference from the scs and gypsy elements may reflect a different mode of action or some degree of specificity for some types of promoters, but most likely the scs′ fragment used lacks some sequences important for effective insulation, when compared to a larger fragment originally used by KELLUM and SCHEDL (1991).

The use of the gypsy conditional insulator showed that two of 12 insertions of transposons containing the PRE are not normally detectable because the *miniwhite* gene marker is completely repressed. Our results using the YGPMG construct suggest that this fraction could be as high as one quarter of the lines recovered. The extreme variability of the variegation displayed by different lines of a given construct confirms the idea that the formation of a silencing complex depends strongly on the chromosomal environment of the PRE-containing transposon. The sequences flanking the insertion site could have a positive or negative effect on the ability of the PRE to repress. Positive effects could be viewed as caused by the presence of sequences that participate in the formation of a stable PcG complex and enhance its ability to repress. Negative effects could be due to chromosomal regions unusually poor in such sequences. Another possible cause of negative effects could be the presence of enhancer elements that acti-

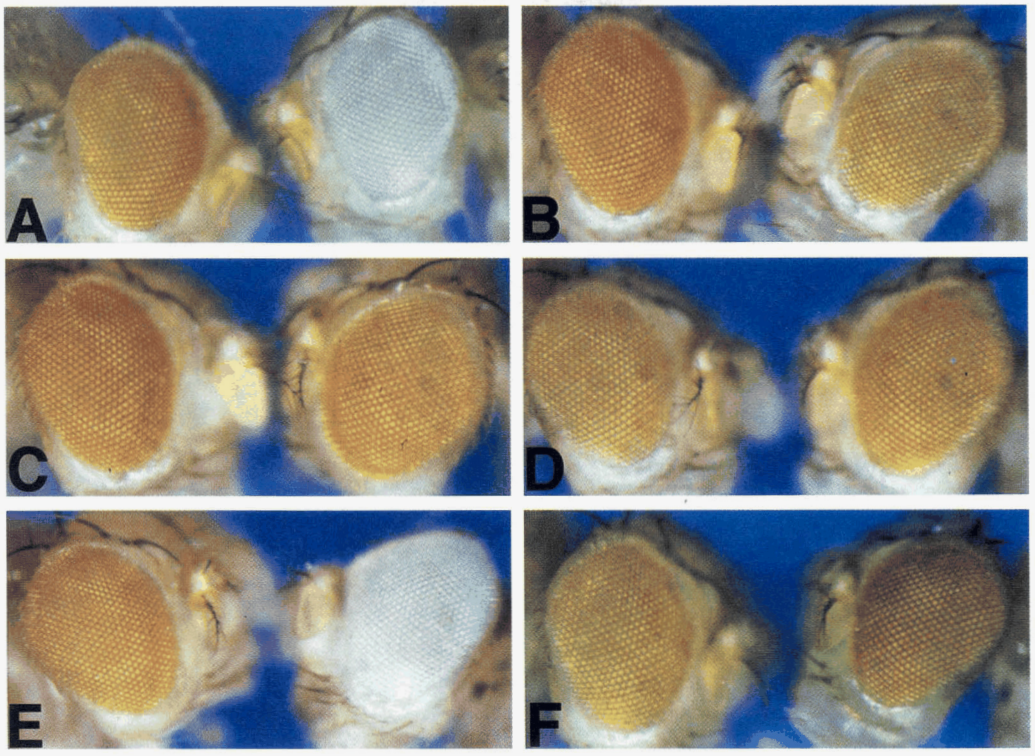

FIGURE 3.—Interactions between different transposons. (A) GPMG-27 males heterozygous (left) or homozygous (right) for the transposon. (B) Heterozygous GPMG-27 male (left) and *trans*-heterozygous males with one copy of GPMG-27 on chromosome *3* and one of GPMG-28 on chromosome *2* (right). (C) A heterozygous GPMG-27 male (left) is reproducibly more pigmented than a male with one copy of GPMG-27 and two copies of GPMG-28 (right). (D) A *trans*-heterozygous male with GPMG-27 and GPMG-28 (left) is equally pigmented as a male with one GPMG-27 and two GPMG-28 (right). (E) Heterozygous GPMG-27 male (left) and male doubly homozygous for both GPMG-27 and GPMG-28 (right). (F) *Trans*-heterozygous male with GPMG-27 and GPMG-28 (left) compared with male with same transposon configuration but with homologous pairing prevented by TM3 and SM5 balancer chromosomes (right).

vate the expression of the *miniwhite* gene at early embryonic stages. We have shown that transcriptional activity of a gene at the time the PcG complex is established in the early embryo interferes with the establishment of silencing (POUX *et al.* 1996). Insulation with a gypsy element on both sides, as in the GPMG construct, would prevent both effects. In fact at least one GPMG line is completely repressed in the homozygous state but shows variegated pigmentation when put in a *su(Hw)*$^-$ background. We suppose that in this case the flanking

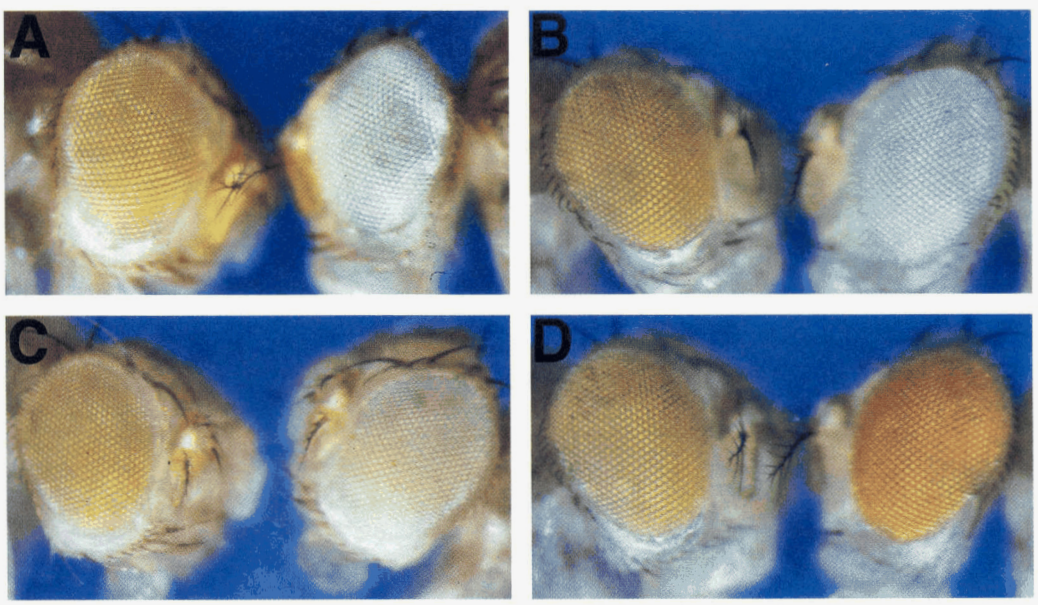

FIGURE 4.—Effect of *su(Hw)* on *trans* interactions. (A) GPMG-28 males heterozygous (left) or homozygous (right) for the transposon. (B) The same configuration in a *su(Hw)*$^-$ background. (C) Male with one copy of GPMG-28 (left) compared with male *trans*-heterozygous for GPMG-28 and GPMG-4 (right). (D) The same configuration in a *su(Hw)*$^-$ background.

sequence stimulates expression and interferes with repression.

The GPMG construct should, in principle, reveal the intrinsic activity of the PRE contained within the transposon, insulated from outside influences. Although the GPMG lines show a very low frequency of variegation as heterozygotes, the YGPMG lines reveal that this is probably due to a high frequency of complete repression. Both constructs show that pairing between two PREs in flies homozygous for the transposon leads to complete repression in a high proportion of the lines. Both sets of lines display two features indicating that the two flanking gypsy elements are not sufficient to afford complete insulation from outside influences. One, the variability in levels of pigmentation, is discussed below. The other is the high variability in the degree of PcG-dependent silencing. Some lines variegate weakly or not at all, others are completely repressed even in the heterozygous state. One possible explanation for this variability invokes early events in the preblastoderm embryo. We do not know when the gypsy element becomes effective in establishing a block, although it appears to be effective already in the blastoderm embryo (CAI and LEVINE 1995). If a transposon is inserted near an enhancer that can activate the *miniwhite* gene before the gypsy barrier is erected, the early expression of the gene might interfere with the establishment of silencing by the PRE (POUX *et al.* 1996). Another explanation that would account also for the variability in the degree of PcG-dependent silencing is suggested by the pairing interactions.

***Trans* interactions and nuclear dynamics:** In both sets of GPMG lines homozygous pairing enhances the PcG repression in some lines but not others, often resulting in complete silencing. It has long been known that, in dipterans, interphase chromosomes tend to be associated with their homologues, providing a substrate for such pairing-dependent interactions as transvection, the *zeste-white* interaction, pairing-enhanced transcription (GOLDSBOROUGH and KORNBERG 1996). The extreme enhancement of PcG silencing that often results in lines homozygous for PRE-containing transposon is another instance and, in fact, it is probably the mechanism at the root of the *zeste-white* interaction (PIRROTTA and RASTELLI 1994; L. RASTELLI and V. PIRROTTA, unpublished observations). Such pairing-enhanced silencing has been observed with other PREs (FAUVARQUE and DURA 1993; CHAN *et al.* 1994; KASSIS 1994; KAPOUN and KAUFMAN 1995) and is related to the phenomenon of transposon "homing," the tendency for PRE-containing transposon to insert in the vicinity of other PRE-containing chromosomal sites (FAUVARQUE and DURA 1993; KASSIS 1994). In both cases the association is most likely due to the interactions between PcG proteins and the cooperative assembly of PcG complexes, facilitated by the proximity of two PREs. This raises the question why such interactions occur in some lines but not in others containing the same transposon insulated by gypsy elements at both ends. One possibility is that the strength of homologous pairing is not the same everywhere along the chromosome and some chromosomal regions are much more likely to pair stably. PREs inserted in such regions would be strongly enhanced by homologous pairing. The insulation from the chromosomal environment in the GPMG constructs would not block such pairing effects.

Another explanation is suggested by the surprising finding that *trans* interactions can occur also between transposons inserted at different sites. Although they are more frequent among transposons on the same chromosome, such interactions also occur between different chromosomes, showing that they are strong enough to bring together two chromosome segments of different provenance. These results imply that some nonhomologous chromosomal regions can associate in the nucleus sufficiently stably to allow the interaction between PREs. Such *trans* interactions are probably also responsible for the high variability not only in the degree of silencing but also in the levels of pigmentation observed among the GPMG or YGPMG lines. Instead of the pale yellow color reported for a doubly blocked *miniwhite* gene by KELLUM and SCHEDL (1991), using the scs insulator, or by ROSEMAN *et al.* (1993), using the gypsy element to insulate the *miniwhite* gene, many of these lines have strong orange or red eye pigmentation. This could be explained if in some cases the *trans* interactions facilitated by the PRE bring the transposon together with a chromosomal region containing transcriptional enhancers active in the eye. This explanation would require that the resulting transactivation overcome the silencing effects of the PRE. Although these results do not prove that transactivation can occur, they suggest that the behavior of an individual PRE depends on the sum total of the interactions with all those remote parts of the genome that are accessible from its chromosomal position.

We do not know what is responsible for such associations between different regions of the genome. Cytological evidence for their existence is provided by the observation of ectopic filaments often connecting different parts of a chromosome or different chromosomes in salivary chromosome spreads. These have sometimes been attributed to sequence homology, for example, provided by copies of transposable elements. Other interactions stabilizing such associations might be those between PREs. Recently, the association between the centric heterochromatin and a block of heterochromatin inserted near the *bw* locus has been shown to accompany the heterochromatic silencing of the *bw* gene in the bw^D mutation (CSINK and HENIKOFF 1996; DERNBURG *et al.* 1996). The interaction between PREs inserted at homologous or heterologous sites shows that associations between silencing complexes at different chromosomal sites are not limited to heterochromatin

and suggests a common mechanism for both phenomena based on interactions between the silencing complexes. According to this, the probability of establishing a repressive chromatin state or the strength or stability of the repressive state are strongly enhanced by the physical proximity to other binding sites for the silencing proteins in the nucleus. The probability that a particular site will come in contact with other sites will depend on the folding of the chromosome arms, which will in turn be dictated by the sum total of the interactions possible between sequences on a chromosome arm or between chromosomes. The clustering together of PRE-containing sites in the genome might be responsible for the formation of the limited number of foci staining with anti-Polycomb antibodies that has been observed in diploid nuclei (MESSMER *et al.* 1992). This does not mean that all PRE sites interact or that the possibility of interaction will automatically induce the silenced state. However, the results suggest that the correct activity of a PRE will in part depend on its chromosomal location and on the possibilities for PRE-PRE interactions that that location affords.

Contribution of the gypsy element: Does the gypsy element contribute to the activity of the PRE beyond its role as an insulator? A comparison of the results obtained with the PGM and GPM lines in the presence or absence of *su(Hw)* function suggests that it has little intrinsic silencing or stimulating activity. The frequency of variegation is not significantly different in the GPM lines with or without *su(Hw)* function and essentially similar to that found in the PGM lines in the *su(Hw)* mutant. Although we cannot exclude the possibility of some residual su(Hw) function in the *su(Hw)* mutant, these results imply that the gypsy insulator has no appreciable influence on PRE repression other than that attributable to its insulator function.

A somewhat different view is provided by the GPMG and YGPMG constructs. The frequency with which these constructs produce the strong pairing interactions that result in complete silencing may be somewhat higher than with the GPM construct or the PGM in the *su(Hw)* mutant (11/19 for GPMG, 6/13 for YGPMG, 7/18 for GPM and 3/9 for PGM, excluding the lines that are heterozygous white or homozygous lethal), yet these interactions are in most cases not affected in the *su(Hw)* mutant. However, in the two cases tested, the *su(Hw)* mutation virtually abolishes the heterologous *trans* interaction. Though other interpretations are not excluded, this suggests that, directly or indirectly, the su(Hw) protein contributes to the interactions, possibly by facilitating pairing. That this contribution is likely to be marginal is indicated by the fact that, in the absence of a PRE, a *miniwhite* gene doubly blocked by two gypsy elements shows little position effect and gives a uniform pale color (ROSEMAN *et al.* 1993). The transposon "homing" phenomenon suggests that PRE interactions in the absence of gypsy elements are sufficient to direct a PRE-containing transposon to the vicinity of other PRE-containing sites. However, the reports of transvection-like effects between *yellow* alleles containing gypsy insertions support the likelihood that the su(Hw) complex in the gypsy element can, in some cases, mediate pairing-dependent interactions (GEYER *et al.* 1990; GEORGIEV and CORCES 1995).

We have not yet detected interactions between nonhomologously inserted PRE-containing transposons in the absence of gypsy but the set of lines tested in this case was smaller than the extensive set of GPMG lines examined in this work. However, interactions between transposons inserted at different sites has been reported also with constructs including the *Mcp* element from the *iab-5* region of the bithorax complex (cited in VAZQUEZ *et al.* 1993; M. MÜLLER, K. HAGSTROM, H. GYURKOVIC and P. SCHEDL, personal communication). It is interesting to note that, in addition to the *Mcp* PRE, these transposons contained the scs′ insulator element at the 3′ end of the *miniwhite* gene, making it analogous in structure to our GPM transposons and suggesting that the insulation of the PRE might enhance its ability to interact with nonhomologous genomic sites. We do not know if contribution of the gypsy element to *trans* interactions is associated with its insulator activity but it is interesting to note that some of the PREs identified in the bithorax complex are closely associated with insulators (HAGSTROM *et al.* 1996).

Insulator action: Little is known about the mechanism of action of insulator elements. The fact that the insulators block the action of silencing elements as well as enhancers accords poorly with the idea that their function is a repressive one, rendered unidirectional by some additional constraint. This view has been advanced for the gypsy element where the interaction of the mod(mdg4) protein with the su(Hw) protein is necessary for insulator function (GERASIMOVA *et al.* 1995). In the absence of mod(mdg4) protein, the gypsy element acts as a bidirectional repressor instead of an insulator in the case of the *yellow* gene. However, at other loci affected by *gypsy* insertions, *mod(mdg4)* mutations result in loss of insulator function rather than bidirectional repression (KIM *et al.* 1993; GEORGIEV and KOZYCINA 1996), suggesting that the specific effect on a gene may depend on the local topology of regulatory elements. In our case, *mod(mdg4)* mutations have an effect similar to, though weaker than that of, *su(Hw)* mutations in releasing the block to PRE silencing.

Recent articles have argued that the gypsy insulator does not function by capturing the distal enhancer because such an enhancer is still free to act upon a second promoter situated adjacent to the enhancer and on the same side of the gypsy barrier (CAI and LEVINE 1995; SCOTT and GEYER 1995). However, these experiments only say that the enhancer is still free to act on a promoter on the same side of the barrier and do not rule out the possibility that the enhancer is captured and

prevented from looping further whenever it attempts to pass across the gypsy barrier. WIJGERDE *et al.* (1995) have shown that an enhancer like the globin LCR can interact alternately with different promoters, implying that such interactions are transient and reversible. Similarly, the interaction between an enhancer and the gypsy element might be transient and not exclusive. The insulator effect could be accounted for if the enhancer is more likely to interact with an intervening gypsy element than with a more distal promoter but is not prevented from interacting with another promoter on the opposite side.

It has been said, in the absence of a better explanation, that the insulator elements act as boundaries between non-intercommunicating chromatin domains (SCOTT and GEYER 1995). Our results certainly indicate that our GPMG or GPM transposons are still able to interact with other chromosome sequences in *trans*. A more accurate description would be that the insulators prevent communication along a chromatin fiber, whether by sliding of proteins or by short range looping. What is difficult to explain is how they could interfere with the long range looping over the tens of kilobases that would be required to explain the block of distant enhancers by *gypsy* in the *cut* gene or in the *Ubx* gene (PEIFER and BENDER 1986; JACK *et al.* 1991; DORSETT 1993). The fact that the insulator elements act on both enhancers and silencers suggests to us the possibility that in both cases a similar scenario might be involved. The silencing effects of a PRE over large distances have been proposed to occur by the recruitment of isolated weak target sequences starting from a nucleation center at the PRE and leading to the spread of the complex by a series of short loops between such weak targets (PIRROTTA and RASTELLI 1994). A similar model has been proposed for the action of yeast silencers (BOSCHERON *et al.* 1996). The same type of mechanism might be imagined to mediate the interaction of distant enhancers with the promoter. Stable binding of an activator at a distant enhancer, favored by cooperative interactions at multiple target sequences, might then recruit weak binding sites, involving isolated, poor consensus binding sequences that would be unable to bind the activator by themselves. Such progressive recruitment of secondary sites would eventually lead the enhancer complex within striking distance of the promoter (Figure 5). This hypothesis finds some support in the findings of WALTER *et al.* (1994), who reported that ftz and eve proteins are found associated with the whole length of target genes and not just with known enhancer sites. This broad distribution of enhancer-binding factors is analogous to that found for the Pc protein at the *Ubx* locus (ORLANDO and PARO 1993). A model explaining action at a distance by a series of short-range loops might permit insulator elements to block both enhancers and silencers by interfering with short-range looping, possibly exploiting the series of sharp bends associated with the su(Hw) binding sites (SPANA and CORCES 1990), while *trans* interactions involving PRE-PRE pairing or promoter activation through transvection would not be affected because they would not necessitate the passage of the PRE through the vicinity of the insulator element.

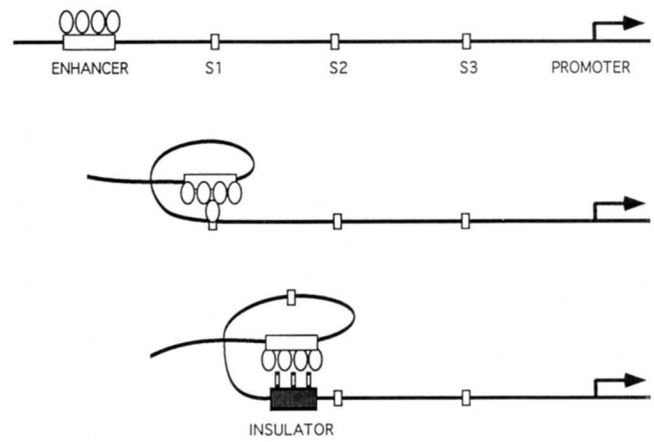

FIGURE 5.—Model for distant enhancer-promoter interactions. The distant enhancer is represented as containing multiple binding sites for enhancer factors. Secondary sites S1, S2 and S3 bind weakly to the enhancer factors. A series of short-range looping interactions with secondary sites brings the enhancer complex progressively nearer to the promoter. The presence of an insulator, whose proteins saturate interactions with the enhancer proteins, blocks further progress of the complex.

We thank TING WU, PAM GEYER, TATIANA GERASIMOVA and GUNTER REUTER for fly mutants, PAM GEYER, FRANÇOIS KARCH and UELI LAEMMLI for clones of the insulator elements and NICOLAS HULO and MARTIN MÜLLER for discussions. This work was supported by a grant from the Swiss National Science Foundation to V.P.

LITERATURE CITED

BOSCHERON, C., L. MAILLET, S. MARCAND, M. TSAI-PFLUGFELDER, S. M. GASSER *et al.*, 1996 Cooperation at a distance between silencers and proto-silencers at the yeast HML locus. EMBO J. **15:** 2184–2195.

CAI, H., and M. LEVINE, 1995 Modulation of enhancer-promoter interactions by insulators in the Drosophila embryo. Nature **376:** 533–536.

CHAN, C. S., L. RASTELLI and V. PIRROTTA, 1994 A Polycomb response element in the *Ubx* gene that determines an epigenetically inherited state of repression. EMBO J. **13:** 2553–2564.

CSINK, A. K. and S. HENIKOFF, 1996 Genetic modification of heterochromatic association and nuclear organization in Drosophila. Nature **381:** 529–531.

DERNBURG, A. F., K. W. BROMAN, J. C. FUNG, W. F. MARSHALL, J. PHILIPS, 1996 Perturbation of nuclear architecture by long-distance chromosome interactions. Cell **85:** 745–759.

DORSETT, D., 1993 Distance-independent inactivation of an enhancer by the *suppressor of Hairy-wing* DNA-binding protein of *Drosophila*. Genetics **134:** 1135–1144.

FARKAS, G., and A. UDVARDY, 1992 Sequence of scs and scs′ *Drosophila* DNA fragments with boundary function in the control of gene expression. Nucleic Acids Res. **20:** 2604.

FAUVARQUE, M.-O., and J.-M. DURA, 1993 *polyhomeotic* regulatory sequences induce developmental regulator-dependent variegation and targeted P-element insertions in *Drosophila*. Genes Dev. **7:** 1508–1520.

GEORGIEV, P. G., and V. G. CORCES, 1995 The su(Hw) protein bound to gypsy sequences in one chromosome can repress en-

hancer-promoter interactions in the paired gene located in the other homolog. Proc. Natl. Acad. Sci. USA **92:** 5184–5188.

GEORGIEV, P., and M. KOZYCINA, 1996 Interaction between mutations in the *suppressor of Hairy wing* and *modifier of mdg4* genes of *Drosophila melanogaster* affecting the phenotype of gypsy-induced mutations. Genetics **142:** 425–436.

GERASIMOVA, T. I., D. A. GDULA, D. V. GERASIMOV, O. SIMONOVA and V. G. CORCES, 1995 A *Drosophila* protein that imparts directionality on a chromatin insulator is an enhancer of position-effect variegation. Cell **82:** 587–597.

GEYER, P. K., and V. G. CORCES, 1992 DNA position-specific repression of transcription by a *Drosophila* zinc finger protein. Genes Dev. **6:** 1865–1873.

GEYER, P. K., M. M. GREEN and V. G. CORCES, 1990 Tissue-specific enhancers may act in *trans* on the gene located in the homologous chromosome: the molecular basis of transvection in *Drosophila.* EMBO J. **9:** 2247–2256

GEYER, P. K., C. SPANA and V. G. CORCES, 1986 On the molecular mechanism of gypsy-induced mutations at the *yellow* locus of *Drosophila melanogaster.* EMBO J. **5:** 2657–2662.

GINDHART, J. G., JR., and T. C. KAUFMAN, 1995 Identification of Polycomb and trithorax group responsive elements in the regulatory region of the *Drosophila* homeotic gene *Sex combs reduced.* Genetics **139:** 797–814.

GOLDSBOROUGH, A. S., and T. B. KORNBERG, 1996 Reduction of transcription by homologue asynapsis in Drosophila imaginal discs. Nature **381:** 807–810.

HAGSTROM, K., M. MÜLLER and P. SCHEDL, 1996 Fab-7 functions as a chromatin domain boundary to ensure proper segment specification by the Drosophila bithorax complex. Genes Dev. **10:** 3202–3215.

HOLDRIDGE, C., and D. DORSETT, 1991 Repression of *hsp70* heat shock gene transcription by the *suppressor of Hairy wing* protein of *Drosophila melanogaster.* Mol. Cell. Biol. **11:** 1894–1900.

JACK, J., D. DORSETT, Y. DELOTTO and S. LIU, 1991 Expression of the *cut* locus in the *Drosophila* wing margin is required for cell type specification and is regulated by a distant enhancer. Development **113:** 735–747.

KAPOUN, A. M., and T. C. KAUFMAN, 1995 Regulatory regions of the homeotic gene *proboscipedia* are sensitive to chromosomal pairing. Genetics **140:** 643–658.

KASSIS, J. A., 1994 Unusual properties of regulatory DNA from the *Drosophila engrailed* gene: three "pairing-sensitive" sites within a 1.6 kb region. Genetics **136:** 1025–1038.

KASSIS, J. A., E. P. VANSICKLE and S. M. SENSABAUGH, 1991 A fragment of engrailed regulatory DNA can mediate transvection of the *white* gene in *Drosophila.* Genetics **128:** 751–761.

KELLUM, R., and P. SCHEDL, 1991 A position-effect assay for boundaries of higher order chromosomal domains. Cell **64:** 941–950.

KELLUM, R., and P. SCHEDL, 1992 A group of scs elements function as domain boundaries in an enhancer-blocking assay. Mol. Cell. Biol. **12:** 2424–2431.

KIM, J., B. SHEN, and D. DORSETT, 1993 The *Drosophila melanogaster* suppressor of Hairy-wing zinc finger protein has minimal effects on gene expression in *Saccharomyces cerevisiae.* Genetics **135:** 343–355.

MAZO, A. M., L. J. MIZROKHI, A. A. KARAVANOV, Y. A. SEDKOV, A. A. KRICHEVSKAJA *et al.,* 1989 Suppression in *Drosophila*: *su(Hw)* and *su(f)* gene products interact with a region of gypsy (mdg4) regulating its transcriptional activity. EMBO J. **8:** 903–912.

MESSMER, S., A. FRANKE and R. PARO, 1992 Analysis of the functional role of the Polycomb chromo domain in *Drosophila melanogaster.* Genes Dev. **6:** 1241–1254.

ORLANDO, V., and R. PARO, 1993 Mapping Polycomb-repressed domains in the bithorax complex using *in vivo* formaldehyde cross-linked chromatin. Cell **75:** 1187–1198.

PEIFER, M., and W. BENDER, 1986 The *antherobithorax* and *bithorax* mutations of the bithorax complex. EMBO J. **5:** 2293–2304.

PIRROTTA, V., 1988 Vectors for P-mediated transformation in *Drosophila*, pp. 437–456, in *Vectors: A Survey of Molecular Cloning Vectors and Their Uses,* edited by R. L. RODRIGUEZ and D. T. DENHARDT. Butterworth, Boston.

PIRROTTA, V., 1995 Chromatin complexes regulating gene expression in *Drosophila.* Curr. Opin. Genet. Dev. **5:** 466–472.

PIRROTTA, V., and L. RASTELLI, 1994 *white* gene expression, repressive chromatin domains and homeotic gene regulation. BioEssays **16:** 549–556.

POUX, S., C. KOSTIC and V. PIRROTTA, 1996 Hunchback independent silencing of late *Ubx* enhancers by a Polycomb Group Response Element. EMBO J. **15:** 4713–4722.

ROSEMAN, R. R., V. PIRROTTA and P. K. GEYER, 1993 The su(Hw) protein insulates expression of the *Drosophila melanogaster white* gene from chromosomal position-effects. EMBO J. **12:** 435–442.

RUBIN, G. M., and A. C. SPRADLING, 1983 Vectors for P element-mediated gene transfer in Drosophila. Nucleic Acids Res. **11:** 6341–6351.

SCOTT, K. S., and P. K. GEYER, 1995 Effects of the su(Hw) insulator protein on the expression of the divergently transcribed *Drosophila* yolk protein genes. EMBO J. **14:** 6258–6279.

SPANA, C., and V. G. CORCES, 1990 DNA bending is a determinant of binding specificity for a *Drosophila* zinc finger protein. Genes Dev. **4:** 1505–1515.

VAZQUEZ, J., and P. SCHEDL, 1994 Sequences required for enhancer blocking activity of scs are located within two nuclease-hypersensitive regions. EMBO J. **13:** 5984–5993.

VAZQUEZ, J., G. FARKAS, M. GASZNER, A. UDVARDY, M. MÜLLER *et al.,* 1993 Genetic and molecular analysis of chromatin domains. Cold Spring Harbor Symp. Quant. Biol. **58:** 45–54.

WALTER, J., C. A. DEVEER and M. D. BIGGIN, 1994 Two homeo domain proteins bind with similar specificity to a wide range of DNA sites in *Drosophila* embryos. Genes Dev. **8:** 1678–1692.

WIJGERDE, M., F. GROSVELD and P. FRASER, 1995 Transcription complex stability and chromatin dynamics *in vivo.* Nature **377:** 209–213.

WU, C.-T. and M. HOWE, 1995 A genetic analysis of the *Suppressor 2 of zeste* complex of *Drosophila melanogaster.* Genetics **140:** 139–181.

ZHAO, K., C. M. HART and K. LAEMMLI, 1995 Visualization of chromosomal domains with boundary element-associated factor BEAF-32. Cell **81:** 879–889.

ZINK, D., and R. PARO, 1995 *Drosophila* Polycomb-group regulated chromatin inhibits the accessibility of a *trans*-activator to its target DNA. EMBO J. **14:** 5660–5671.

ZINK, B., Y. ENGSTRÖM, W. J. GEHRING and R. PARO, 1991 Direct interaction of the Polycomb protein with *Antennapedia* regulatory sequences in polytene chromosomes of *Drosophila melanogaster.* EMBO J. **10:** 153–162.

Communicating editor: S. HENIKOFF